New claims 46 and 54 are related to existing composition and method claims, and are thus supported in the specification as filed.

The changes made to the claims by the foregoing amendments, including **[deletions]** and insertions, are shown on an attached sheet entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**, which follows the signature page of this Amendment. Applicants submit the above amendments do not constitute the addition of new matter.

As a result of this amendment, claims 1-3, 11-21, 30, 31, 33, 34, 36, 37, and 39-54 are pending for examination. The following remarks address the substance of the Office Action:

I. Status of the claims

Applicants acknowledge that claims 1-3, 11-21, 30, 31, 33, 34, 36, 37, and 39-45 are pending in the application. Although claim 32 does not constitute a pending claim, this claim and claim 33 have been formerly cancelled since these claims depend, either directly or indirectly, from previously cancelled claim 22 (see the response to Paper No. 12).

Applicants also acknowledge that claims 12, 14, 15, 33, 34, 36, 37, and 39-41 have been withdrawn from consideration. Claim 33 has been cancelled, as noted above. However, claim 41 is a Group I claim and in previous Office Actions has been included amongst the claims under examination (see, for example, Paper No. 12). Applicants assume that claim 41 has been classified erroneously as a withdrawn claim in the present Action and respectfully request that it be examined during the Examiner's consideration of this response.

With regard to claims 12, 14, 15, 36, 37, 39 and 40, Applicants remind the Examiner that in the response to Paper No. 5, rejoinder of the Group III claims was requested on allowance of the Group I claims and claim 1 in particular. Each of claims 12, 14, 36, 37, 39 and 40 is dependent, either directly or indirectly, on claim 1 and (save for claim 34) relate to pharmaceutical compositions comprising the antimicrobial proteins of the invention, or methods of using the antimicrobial proteins on mammals. Applicants note that claim 31 as amended by the response to Paper No. 12 has been rejoined. This claim is directed to a composition comprising a protein fragment of an antecedent claim together with a pharmaceutically-acceptable carrier diluent or excipient. Applicants respectfully submit that rejoinder of claims 12, 14, 36, 37, 39 and 40 is warranted in view of the rejoinder of claim 31.

Concerning claim 34, this was withdrawn from consideration following amendment made as part of a response to Paper No. 10. An amendment made to the claim in Paper No. 14 brought the claim into conformity with other Group I method claims such as claim 13. However, claim 34 still stands withdrawn. Further amendment of the claim has been made in this response and Applicants respectfully request that it be examined during the Examiner's consideration of the response.

II. Objections and rejections concerning the phrase "...other than cysteine..."

Introductory Comments

In paragraphs 4, 6, 8 and 9 of the Action, the Examiner has objected to the passage "X is any amino acid residue other than cysteine". The various objections stem from the Examiner's belief that the words "other than cysteine" constitute new matter. In rejecting argument included in Applicants' response to the previous Office Action, the Examiner makes the following statement:

> "Applicant's invention cannot be read into the claims or the disclosure, what is disclosed or recited is what is considered during examination."

Applicants respectfully submit that there is a clear disclosure of X being any amino acid other than cysteine and any reasonable consideration of the description would lead one of even less than ordinary skill in the art to this conclusion.

The Examiner asserts that Applicants' contention that support for the "other than cysteine" terminology can be found at page 10 of the specification is not accurate since there is not a specific recitation to this effect. Applicants do not deny that there is an absence of such an explicit recitation in the description. However applicants also point out that the disclosure as a whole must be considered.

Support for "X is any amino acid other than cysteine"

Applicants respectfully submit that the specification as filed does provide support for the claimed limitation "X is any amino acid other than cysteine." The decision in *Ex parte Parks (Bd Pat App & Inter, 30 U.S.P.Q. 2d 1234 (1993))* allows for the requirements of the first paragraph of 35 U.S.C. §112 to be satisfied if the originally-filed disclosure would have conveyed to one having ordinary skill in the art that Applicants had possession of the claimed subject matter,

even if a particular concept was not literally stated in the originally filed application. This is the case in the presently-claimed invention.

Beginning on page 4 and appearing again on page 10 are polypeptide formulations contemplated in the present invention. Included in the descriptions of these formulations is the phrase "...relative cysteine spacing..." *see*, page 4, lines 21-29, *. In addition, the embodiments and aspects of the presently claimed invention are described in reference to the spacing and/or distribution of cysteine residues, *see* for example:

"...to achieve substantially the same distribution of positively charged residues and cysteine residues..." (*see* page 5, lines 32-33);

"...could not be altered without seriously affecting the structure...is the content and spacing of the cysteine residues..." (*see* page 10, lines 6-20);

"...retain the distribution of positively charged residues relative to cysteine residues as found in MiAMP2 proteins..." (*see* page 13, lines 25-34); "...contain two sets of CXXXC motifs which are important in stabilising the three-dimensional structure of the protein..." (*see* page 14, lines 6-14); and

"...the cysteine side chains in one helix must form covalent bonds with the cysteine side chains from each respective helix..." (*see* page 20, lines 6-21). Within the description on page 20, lines 6-21 (Example 8) is an analysis of the amino acid sequence and descriptions of aspects of the amino acid backbone needed to form the desired helix-turn-helix motifs. The Examiner does not seem to dispute that there is recitation of a polypeptide with a cysteine spacing of C-3X-C-nX-C-3X-C where n is 11 or 12. This in fact cannot be disputed for there is a recitation which includes the foregoing formula at page 4, lines 26 and 27 of the International Application as filed and published under the number "WO 98/27805". Applicants stress that this formula and the passage at page 10 of the specification relate to a specific spacing of cysteine residues. By definition therefore, the cysteine residues of the polypeptide must be spaced apart by other residues to achieve the specified spacing.

Also provided within the description are different residues that may be able to substitute for different positions of polypeptide in the presently claimed invention are discussed on, for example, pages 10 (line 31) to 11 (line 12)

Applicants respectfully submit that included throughout the entire specification is an explicit understanding that the presently claimed invention involves polypeptides with specific

cysteine residue spacings. Formulations are given on pages 4 and 10 of polypeptides contemplated within the invention that do describe the claimed limitation that "X is any amino acid residue other than cysteine.". Thus, in view of the disclosure, Examples and submitted Sequence Listing, it is clear that "X is any amino acid residue other than cysteine" is provided for in the specification as filed.

Inconsistencies within the interpretation "X is any amino acid"

The Examiner contends that the recitation "X is any amino acid" must be interpreted as meaning "any amino acid including cysteine". Applicants submit that this interpretation is inconsistent, *inter alia*, with the disclosed formula and the passage at page 10. If the Examiner's interpretation is applied to "X is any amino acid" including cysteine, the formula could exist as follows:

C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-oC-C-C-C-C-C

where "o" is 1 or 2. The polypeptide is thus a continuous string of cysteine residues with a spacing of C-C. This is clearly not consistent with the spacing called for in the specification, *see* page 10, line 10-16.

Or with X as any amino acid including cysteine, the following formula would also be possible:

C-3X-C-3X-C-3X-C-2X-oX-C-3X-C

where "o" is defined above. Again, the cysteine spacing called for in the specification is destroyed if X is any amino acid including cysteine. To interpret the foregoing passage as meaning that X can be any amino acid residue including cysteine completely nullifies the specified spacing of the subject polypeptides

It is therefore fundamental to the definition of the polypeptides given in the description that to achieve the specified cysteine spacing the residues between the cysteines cannot be cysteine. Thus, as discussed at length above, Applicants submit there is an implicit disclosure within the specification of X being any amino acid residue other than cysteine as recited in claim 1.

The following addresses specific comments on the matters raised in the instant Action:

Objection to the specification

In paragraphs 4, and 6 of the Action, the Examiner has objected to the passage "X is any amino acid residue other than cysteine" as recited in amended claim 1. The various objections stem from the Examiner's belief that the words "other than cysteine" within the claims do not have antecedent basis within the disclosure and that their addition constitutes the addition of new matter into the disclosure.

Applicants have amended the specification on pages 4 and 10 to specify that "X is any amino acid residue other than cysteine." The limitation was fully supported within the disclosure as filed and as described in the above, however, in order to provide the antecedent basis as discussed in Paragraph 4 of the Office Action, Applicants have additionally amended the specification to explicitly provide the antecedent basis for the claimed subject matter. As discussed above and in the following "*Rejection under 35 U.S.C. § 112, first paragraph*" remarks, Applicants respectfully submit that no new matter has been added herewith.

Rejection under 35 U.S.C. §112, first paragraph

The Examiner has rejected claims 1-3, 11, 13, 16-21, 30, 31 and 41-45 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in such a way as to reasonably convey to one of skill in the art that the inventors had possession of the invention at the time the invention was made. With regard to claim 1 and claims dependent thereon, the Examiner asserts that new matter has been added to the first-mentioned claim by the recitation that "X is any amino acid residue other than cysteine". In a reiteration of objections taken under other grounds, the Examiner asserts that the specification provides no support for this amendment because it is disclosed therein that "X in any amino acid residue".

Applicant notes that absent any amendment to the specification, the requirements of the first paragraph of 35 U.S.C. §112 are satisfied by the specification as filed with respect to the recitation that "X is any amino acid residue other than cysteine". In particular, Applicant notes that as decided in *Ex parte Parks (Bd Pat App & Inter, 30 U.S.P.Q. 2d 1234 (1993))*, the requirements of the first paragraph of 35 U.S.C. §112 are satisfied if the originally-filed disclosure would have conveyed to one having ordinary skill in the art that Applicants had possession of the claimed subject matter, even if a particular concept was not literally stated in the originally filed application. In particular, in *Ex parte Parks* the Board of Patent Appeals and Interferences found that the written description requirement was satisfied with respect to a claim limitation specifying that the claimed process was performed "in the absence of a catalyst"

despite the fact that those exact words did not appear in the specification as filed because the originally filed specification conveyed to those skilled in the art that applicant had possession of the concept of performing the method in the absence of a catalyst.

In the present application, the originally filed specification conveys that "X is any amino acid residue other than cysteine," for reasons discussed above. In particular, it is clear that the passage must be read as "X is any amino acid residue other than cysteine" because it deals with the cysteine spacing of the subject polypeptides. One skilled in the relevant art on reading the description would immediately appreciate that a particular cysteine spacing is necessary in a polypeptide for it to have the antimicrobial activity of the polypeptides the subject of the instant application. That skilled person would also appreciate that the inclusion of cysteine as an X residue would destroy the specified spacing of cysteine residues and this has been illustrated in the above remarks regarding the inconsistencies with accepting the interpretation that cysteine is included with "X is any amino acid." The skilled person would thus implicitly read into "X is any amino acid residue" the additional words *"other than cysteine"*. In addition, further evidence that this person of skill in the art would read the passage "X is any amino acid residue", was provided in the Declaration by Dr. Donald Maclean which accompanied the response to the previous Action. In particular, Applicants draw the Examiner's attention to paragraph 6 of the Maclean declaration where the following is stated:

> *In my opinion, therefore, it is implicit within the passage that "X is any amino acid residue" must be read as "X is any amino acid residue other than cysteine".*

Therefore, Applicants submit that the subject matter of claim 1 and dependent claims was described in the specification in such a way as to reasonably convey to one skilled it the relevant art that the inventors had possession of the invention at the time the application was made. Further, Applicants have amended the disclosure to recite that "X is any amino acid other than cysteine," thereby providing the necessary description in the specification for the presently claimed invention.

In view of the foregoing comments and relevant claim amendments, Applicants respectfully request withdrawal of the 35 U.S.C. 112, first paragraph, rejection of claims 1-3, 11, 13, 16-21, 30, 31 and 41-45.

Rejection under 35 U.S.C. §112, second paragraph

The Examiner has rejected claims 1-3, 11, 13, 16-18, 20, 21 and 41-45 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

With regard to claims 1, 2, 11, 13 and 17-21, the Examiner asserts that there is no support in the specification for the phase "X is any amino acid other than cysteine" recited in at least claim 1. As discussed above, the "other than cysteine" recitation is fully supported by the description and an amendment has furthermore been made to include a specific recitation to the foregoing effect in the description. Since there is support for the "other than cysteine" recitation in the description there is an antecedent basis in claims 1, 2, 11, 13 and 17-21 for the phase referred to by the Examiner.

Therefore, Applicants submit that the recitation of the phrase "other than cysteine" in claims 1-3, 11, 13, 16-18, 20, 21 and 41-45 is definite and respectfully request withdrawal of the rejection to the claims under 35 U.S.C. § 112, second paragraph.

III. Additional objections to the specification

In addition to objecting to "X is any amino acid other than cysteine", the Examiner has objected to the specification due to an informality on page 26. Applicants submit that the amendments made on page 26 address the misspelling of "against," thereby rendering the objection inapplicable.

IV. Rejection Under 35 U.S.C. §101

The Examiner has rejected claims 13 and 43-45 under 35 U.S.C. 101 as being directed to non-statutory subject matter. The Examiner asserts that these claims, which are directed to methods of treating microbe infested plants, have no method steps *per se*.

The subject claims have been amended in accordance with the Examiner's suggestion. That is, the claims now recite the steps of administering an effective amount of a compound or composition and for a time that is sufficient to reduce the number of microbes infesting the plant. In anticipation of the Examiner's consideration of claim 34 as examinable, this claim has been amended in the same manner as claims 13 and 43-45.

In view of the positive recitation of steps in the subject method claims, Applicants respectfully submit that the claims are directed to statutory subject matter and respectfully request withdrawal of the rejection to the claims under 35 U.S.C. § 101.

V. Additional claim rejections under 35 U.S.C. § 112, first paragraph

The Examiner has rejected claim 16 under 35 U.S.C. § 112, first paragraph on the assertion that the recitation of the term "known sequence" in the claim method provides no indicia as to what sequence, to whom the sequence is known or how to identify this sequence. Amended claim 16 includes "...searching a sequence database using a suitable algorithm to identify an amino acid sequence which forms a helix-turn-helix structure..." which is supported by the specification as filed in Example 8. Therefore, there is sufficient description and enablement for amended claim 16 as required by 35 U.S.C. § 112, first paragraph.

The Examiner also states in paragraph 8 that the claims are directed to methods of controlling microbial but do not provide any method steps to enable one skilled in the art to practice the claimed invention. While not agreeing with this assertion, Applicants have amended claims 13, 34 and 43-45 to make it abundantly clear where the steps in the subject methods lie. In connection with the Examiner's comment that the specification does not provide sufficient guidance as to a method to control microbial infestation, claims 13, 34 and 34-45 have been amended to recite reduction of microbial infestation.

In light of the above remarks, Applicants respectfully request withdrawal of the rejection to the claims under 35 U.S.C. § 112, first paragraph.

VI. Additional claim rejections under 35 U.S.C. § 112, second paragraph

The Examiner has pointed out that claim 3 is indefinite because the claim does not recite proper Markush language. Applicants submit amended claim 3 recites proper Markush language.

The Examiner states in paragraph 9 that claim 13 is indefinite because:

(a) the claim is a method claim which improperly depends from a product claim;

(b) no positive method steps are recited; and

(c) the end result of "reduction of microbes" is inconsistent with the preamble of the claim.

With regard to (a), claim 13 depends from claim 1 which is directed to a protein fragment *per se*. The latter claim is therefore to a compound, not to a product as asserted by the Examiner. Claim 13, which is to a method can therefore depend from claim 1 since the dependency merely incorporates the compound of the latter claim into the method.

As noted elsewhere, method claims including claim 13 have been amended to ensure the positive recitation of method steps, rendering the rejection of (b) inapplicable.

Concerning item (c) of the rejection to claim 13, the preamble of the claim now reads "a method of reducing the number of microbes infesting a plant". The end result of "reduction of microbes" is now consistent with the claim preamble.

The Examiner has rejected claim 16 because of the "identifying in a known sequence" recitation. As noted above, this recitation is no longer present in the claim as amended. Thus, while not agreeing that the claim was indefinite, Applicants respectfully submit that the rejection is no longer relevant. In connection with claim 16, the Examiner states that "the claim is also indefinite as it is not apparent which residues would or would not be substituted and in what positions". Applicants assume that this part of the rejection is directed at step (b) of the claimed method. The same objection was taken in the previous Office Action (Paper No. 12). Comments on the objection were included in a response to Paper No. 12 and for the Examiner's benefit, a reiteration of these comments follows. To aid the Examiner's consideration of the comments, the relevant portion of claim 16 is hereafter reproduced.

b) substituting individual residues in said amino acid sequence to achieve a sequence having the same distribution of positively charged residues and cysteine residues as the distribution found in a protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 5.

A person of ordinary skill in the art would immediately appreciate on considering the claim that the foregoing step involves comparing the sequence (the test sequence) having a helix-turn-helix structure with SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 5. This comparison would allow identification of the residues in the test sequence corresponding to positively charged and cysteine residues in the reference sequences. The skilled person would then immediately know which residues would have to be substituted to give the same distribution of positively charged and cysteine residues in the test sequence as in the reference sequences.

Contrary to the Examiner's assertion therefore, Applicants believe that the claim is not indefinite as it would be apparent to one of ordinary skill in the art the residues which should or should not be substituted.

The Examiner has rejected claims 43-45 as being indefinite because:

i) claim 43 recites controlling microbial infestation of a plant but the end result is inhibition of microbial infestation;

ii) there is recitation of "infestation of a plant" rather than "infestation in a plant"; and

iii) there are no positive method steps recited.

With regard to (i) above, as with claim 13, claim 43 has been amended to recite a method of reducing microbial infestation of a plant with the end result being the reduction of the number of microbes infesting the plant. The preamble and end result are now consistent.

Concerning item (ii), the amended claims now recite "microbes infesting a plant" thus avoiding the "infestation of a plant" wording.

As noted above, all relevant method claims have been amended to recite the steps of administering an effective amount of a compound or composition and for a time effective to reduce the number of the infesting microbes. The item (iii) objection is consequently no longer relevant to claims 43-45.

In the light of the above comments and the relevant claim amendments, Applicants submit the withdrawal of the 35 U.S.C. 112, second paragraph, rejection of claims 1-3, 11, 13, 16-18, 20-21 and 41-45 is called for.

VII. Rejection under 35 U.S.C. §102(a)

Tartar et al.

In paragraph 10 of the instant Action, the Examiner has maintained the rejection of claim 1 under 35 U.S.C. 102(a) as being anticipated by Tartar *et al.* (EP 0 093 652). The basis of the rejection is that the citation discloses peptides used to vaccinate against *E. coli* enterotoxins and the sequence contained in SEQ ID No: 39 with the formula C3XC12XC3XC wherein X is any amino acid residue and C is cysteine.

As discussed in the response to the previous Office Action (see pages 10 and 11 of Paper No. 14), the rejection based on Tartar *et al.* can only hold if X as defined in claim 1 can be any amino acid residue. However, in the light of what is described as the invention and

particularly in the light of claim 1 as amended, X is clearly any amino acid residue other than cysteine.

With cysteine excluded as an X residue, the Tartar *et al.* sequence of CELCCNPACAGCYNTFYCCELC no longer falls within the formula C3XC12XC3XC. To do so, the Tartar *et al.* sequence would have to be CEL*CNPA*AG*YNTFYC*ELC where each asterisk donates a residue other than cysteine. This would be a vastly different sequence to that actually disclosed.

Applicants have pointed out above that there is support in the description for the "other than cysteine" qualification in the definition of X. Consequently, the amendment to include this qualification in the claim should be allowed. However, as argued elsewhere (see Paper No. 14), the definition of X without the qualification inherently excludes cysteine in view of the specified cysteine spacing.

Since the Tartar *et al.* sequence does not fall within the scope of claim 1 when the proper definition of X is applied, the citation is not an anticipation of the presently claimed invention. Accordingly, Applicants respectfully request that the 35 U.S.C. 102(a) rejection of claim 1 on the basis of Tartar *et al.* be withdrawn.

Voerman

In paragraph 11 of the Action, the Examiner has maintained the rejection of claim 1 under 35 U.S.C. 102(a) as being anticipated by Voerman (WO 96/13585). The Examiner states that Voerman discloses the sequence contained in SEQ ID No: 37 "with 100% sequence identity where the sequence is C3XC10XC3XC".

The formula of amended claim 1, there are 11 or 12 X residues between the two C3XC motifs. The Voerman sequence has 10 X residues between the motifs. Consequently, claim 1 as amended is not anticipated by Voerman. Applicants therefore respectfully request that the rejection of claim 1 under 35 U.S.C. 102(a) be withdrawn.

VIII. Conclusion

The Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. In light of these amendments and remarks, reconsideration and withdrawal of the outstanding rejections is respectfully requested. Should

there be any further questions regarding the above-captioned patent application, the Examiner is respectfully requested to contact the undersigned attorney at the telephone number below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 16 January 2003

By: Megan McCoy

Megan McCoy
Registration No. 51,345
Agent of Record
Customer No. 20,995
(805) 547-5580

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

On page 4, the paragraphs beginning at line 22 and ending at line 27 have been amended as follows:

(iii) a polypeptide containing a relative cysteine spacing of C-2X-C-3X-C-(10- 12)X-C-3X-C-3X-C wherein X is any amino acid residue other than cysteine, and C is cysteine;

(iv) a polypeptide containing a relative cysteine and tyrosine/phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z wherein X is any amino acid residue other than cysteine, and C is cysteine, and Z is tyrosine or phenylalanine;

(v) a polypeptide containing a relative cysteine spacing of C-3X-C-(10-12)X-C-3X-C wherein X is any amino acid residue other than cysteine, and C is cysteine;

On page 10, the paragraph beginning at line 4 has been amended as follows:

It will be appreciated that one skilled in the art could take a protein with known structure, alter the sequence significantly, and yet retain the overall three-dimensional shape and antimicrobial activity of the protein. One aspect of the structure that most likely could not be altered without seriously affecting the structure (and, therefore, the activity of the protein) is the content and spacing of the cysteine residues since this would disrupt the formation of disulfide bonds which are critical to a) maintaining the overall structure of the protein and/or b) making the protein more resistant to denaturation and proteolysis (stabilizing the protein structure). In particular, it is essential that cysteine residues reside on one face of the helix in which they are contained. This can best be accomplished by maintaining a three-residue spacing between the cysteine residues within each helix, but, can also be accomplished with a two-residue interval between the cysteine residues -provided the cysteines on the other helical segment are separated by three residues (i.e., C-X-X-C-X-X-C-nX-C-X-X-X-C-X-X-X-C where C is cysteine, X is any amino acid other than cysteine, and n is the number of residues forming a turn between the two α-helical segments). Aromatic tyrosine (or phenylalanine) residues can also function to add stability to the protein structure if they are located on the same face of the helix as the cysteine side chains. This can be accomplished by providing appropriate spacing of two or three residues between the aromatic residue and the proximate cysteine residue (i.e., Z-X-X-C-X-X-X-C-nX-C-X-X-X-C-X-X-X-Z where Z is tyrosine or phenylalanine).

On page 13, the paragraph beginning at line 23 has been amended as follows:

As indicated above in the description of the **[tenth]**twelfth embodiment, the invention includes within its scope the preparation of antimicrobial proteins based on the prototype MiAMP2 series of proteins. New sequences can be designed from the MiAMP2 amino acid sequences which substantially retain the distribution of positively charged residues relative to cysteine residues as found in the MiAMP2 proteins. The new sequence can be synthesised or expressed from a gene encoding the sequence in an appropriate host cell. Suitable methods for such procedures have been described above. Expression of the new protein in a genetically engineered cell will typically result in a product having a correct three-dimensional structure, including correctly formed **[disulphide]**disulfide linkages between cysteine residues. However, even if the protein is chemically synthesised, methods are known in the art for further processing of the protein to break undesir**[e]**able disulfide bridges and form the bridges between the desired cysteine residues to give the desired three-dimensional structure should this be necessary.

The paragraphs beginning at line 4 and ending at line 15 on page 26 have been amended as follows:

SDS-PAGE gel analysis of the MiAMP2a, b, and d fragment purification is shown in the second panel of Figure 9. Lane contents are as follows: lane 1, molecular weight markers; lane 2, MiAMP2a pre-induced cellular extract**[p]**; lane 3, MiAMP2a IPTG induced cellular extract; lane 4, MiAMP2a Ni-NTA non-binding fraction; lane 5, MiAMP2a elution from Ni-NTA; lane 6, MiAMP2b pre-induced cellular extract; lane 7, MiAMP2b IPTG induced cellular extract; lane 8, MiAMP2b Ni-NTA non-binding fraction; lane 9, MiAMP2b elution from Ni-NTA; lane 10, MiAMP2d pre-induced cellular extract; lane 11, MiAMP2d IPTG induced cellular extract; lane 12, MiAMP2d Ni-NTA non-binding fraction; and lane 13, MiAMP2d elution from Ni-NTA.

Using the vectors described in Example 10, MiAMP2c, and 5 homologues (i.e., MiAMP2a, MiAMP2b, MiAMP2d, TcAMP1 and TcAMP2) were all expressed, purified and tested for antimicrobial activity. The approach taken above can be applied to all of the antimicrobial fragments described in Figure 4. Purified fragments can then be tested for specific inhibition against microbial pathogens of interest.

In the claims:

3. **(Amended Three times)** An isolated or purified protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, **[or]** and SEQ ID NO: 5.

11. **(Amended Three times)** A composition comprising the **[antimicrobial]** protein fragment of claim 1 together with an agriculturally-acceptable carrier diluent or excipient.

13. **(Amended Four Times)** A method of **[controlling microbial infestation of a plant by]** reducing the number of microbes infesting a plant, the method comprising **[treating]** administering to said plant **[with an antimicrobial protein according to]** an effective amount of the protein fragment of claim 1 **[in an amount effective]** for a period sufficient to reduce the number of said microbes.

16. **(Amended Five Times)** A method of preparing an antimicrobial protein, said method comprising:

a) **[identifying in a known]** searching a sequence database using a suitable algorithm to identify an amino acid sequence which forms a helix-turn-helix structure or designing an amino acid sequence which forms a helix-turn-helix structure;

b) substituting individual residues in said amino acid sequence to achieve a sequence having the same distribution of positively charged residues and cysteine residues as the distribution found in a protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5;

c) synthesizing chemically or expressing by recombinant DNA techniques in liquid culture an antimicrobial protein comprising said substituted amino acid sequence; and

e) isolating said antimicrobial protein.

17. **(Amended Three times)** The protein fragment of claim 1, wherein said protein fragment is a polypeptide **[containing]** comprising a **[relative]** cysteine and tyrosine or phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z (SEQ ID NOS: 34-36) wherein **[X is any amino acid residue, and C is cysteine]** X and C are as defined in claim 1, and Z is tyrosine or phenylalanine.

18. **(Amended Three times)** The protein fragment of claim 1, wherein said **[relative]** cysteine spacing comprises C-2X-C-3X-C-(10-12)X-C-3X-C-**[2]**3X-C (SEQ ID NOS:

31-33) wherein **[X is any amino acid residue, and C is cysteine]** X and C are as defined in claim 1.

30. **(Amended Three times)** A composition comprising **[a]** the protein fragment **[according to]** of claim 19 together with an agriculturally-acceptable carrier diluent or excipient.

31. **(Amended Three times)** A composition comprising **[a]** the protein fragment **[according to]** of claim 19 together with a pharmaceutically-acceptable carrier diluent or excipient.

34. **(Amended Three times)** A method of **[controlling microbial infestation of]** reducing the number of microbes infesting a plant, the method comprising **[treating]** administering to said plant **[with]** an effective amount of the composition **[according to]** of claim 11 for a period sufficient to **[inhibit microbial infestation of the plant]** reduce the number of said microbes.

43. **(Amended)** A method of **[controlling microbial infestation of]** reducing the number of microbes infesting a plant, the method comprising **[treating]** administering to said plant **[with]** an effective amount of the composition **[according to]** of claim 30 for a period sufficient to **[inhibit microbial infestation of the plant]** reduce the number of said microbes.

44. **(Amended)** A method of **[controlling fungal infestation of a plant by]** reducing the number of fungi**[,]** infesting a plant, the method comprising **[treating]** administering to said plant **[with]** an effective amount of an antimicrobial protein **[in an amount effective]** for a period sufficient to reduce the number of said fungi, wherein:

said antimicrobial protein comprises a polypeptide comprising a cysteine spacing of C-3X-C-(10-12)X-C-3X-C (SEQ ID NOS: 37-39) wherein X is any amino acid residue, and C is cysteine.

45. **(Amended)** A method of **[controlling fungal infestation of]** reducing the number of fungi infesting a plant, the method comprising **[treating]** administering to said plant **[with]** an effective amount of a composition comprising an antimicrobial protein together with an agriculturally-acceptable carrier diluent or excipient for a period sufficient to reduce the number of said fungi, wherein:

said antimicrobial protein comprises a polypeptide comprising a cysteine spacing of C-3X-C-(10-12)X-C-3X-C (SEQ ID NOS: 37-39) wherein X is any amino acid residue, and C is cysteine.

O:\DOCS\MCM\MCM-3198.DOC